(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 347 864 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89111246.8

(22) Date of filing: 20.06.89

(51) Int. Cl.⁴: A61K 33/34 , A61K 33/30 , A61K 31/525 , A61K 31/455 , A61K 31/375 , A61K 31/105 , A61K 31/095 , A61K 31/68 , A61K 31/505 , A61K 31/205 , A61K 31/00

(30) Priority: 24.06.88 ZA 884556

(43) Date of publication of application: 27.12.89 Bulletin 89/52

(84) Designated Contracting States: AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: Strydom, Andries Johannes Cornelus
21 Dersley Street Bayswater
Bloemfontein Orange Free State(ZA)

(72) Inventor: Strydom, Andries Johannes Cornelus
21 Dersley Street Bayswater
Bloemfontein Orange Free State(ZA)

(74) Representative: Lewinsky, Dietrich, Dipl.-Ing., Dipl.-oec. publ. et al
Patentanwälte Dipl.-Ing., Dipl.-oec. publ.
Dietriech Lewinsky Dipl.-Ing Reiner Prietsch
Gotthardstrasse 81
D-8000 München 21(DE)

(54) Anti-atherogenic agents.

(57) As an anti-atherogenic agent, a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

EP 0 347 864 A2

to form X-S-S-Y within the body by oxidation, or the substance may be administered initially in the form X-S-S-Y. Each of the three forms, viz X-SH, Y-SH and X-S-S-Y is capable of reacting with homocysteine (or cysteine) in the body (or plasma) to form, for example, mixed disulfides of the formula $H_2NCH(COOH)$-CH_2CH_2-S-S-X and/or $H_2NCH(COOH)CH_2CH_2$-S-S-Y. Suitable sulfhydryl and/or disulfide containing compounds are believed to include cystamine, cysteamine, allyl-propyl disulfide, diallyl disulfide, glutathione (oxidized or reduced) and even cyst(e)ine, or the like.

Instead, or additionally, the substance or composition may be, or include, at lease one compound capable of decreasing homocysteine and/or cysteine levels per se in plasma, by its function as a co-factor or co-substrate in the transsulfuration pathway or methionine metabolism. Examples of such compounds are Vitamin B_6, Vitamin B_{12}, folic acid, co-substrates such as betaine and choline, or their metabolic precursors.

Still further, the substance or composition may be, or include, salts like magnesium chloride and/or at least one compound from the group of substances known as bioflavonoids (or flavonoids) like hydroxyethylrutoside and/or at least one compound from the group of substances that have a vasodilatory or antivasoactive action, or for this matter, arterial cell relaxation action, for example, a substance like cilazapril, an angiotensin converting enzyme (ACE) inhibitor, ie all of them substances that are believed to react directly or indirectly with relevant arterial wall cells and/or relevant structural/functional proteins of the arterial wall cell-cement, so as to effectively reduce directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds of the relevant structural/functional proteins like fibronectin in the arterial wall cell-cement, thereby protecting them from the deleterious disulfide exchange reactions with homocysteine and/or cysteine, as described above.

The substance or composition may be administered on its own, or in admixture with a pharmaceutically acceptable adjunct, and can typically be administered orally, in which case it can be in the form of a tablet including a suitable binder or carrier, capsule, syrup including a suitable base, or the like.

It can be administered at any desired dosage rate, within the so-called 'therapeutical window' range. For example, it can be administered at relatively low dosages to healthy persons having "normal" homocysteine and/or cysteine levels, eg prophylactic or preventative dosages, or at relatively high therapeutic dosages to homocystinuric persons. For example, folic acid can be administered at a daily dosage rate of about 5 milligram ("mgm"), pyridoxine hydrochloride (Vitamin B_6) at a daily rate of about 50 mgm, cyanocobalamin (Vitamin B_{12}) at a daily rate of about 0,05 mgm, and choline or betaine at a daily rate of about 5 gm.

According to a fourth aspect of the invention, there is provided the use of an active substance or composition capable of lowering, in plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby to inhibit directly atherosclerosis in the human body, for the manufacturing of a pharmaceutical to inhibit atherosclerosis.

According to a fifth aspect of the invention, there is provided the use of an active substance or composition capable of inhibiting, ie hindering or lowering, the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby directly inhibiting atherosclerosis in the human body, for the manufacturing of a pharmaceutical to inhibit atherosclerosis.

The invention extends also to the use of the combination of (i) an active substance or composition capable of lowering, in plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, and (ii) an active substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby directly inhibiting atherosclerosis in the human body, for the manufacturing of a pharmaceutical to inhibit atherosclerosis.

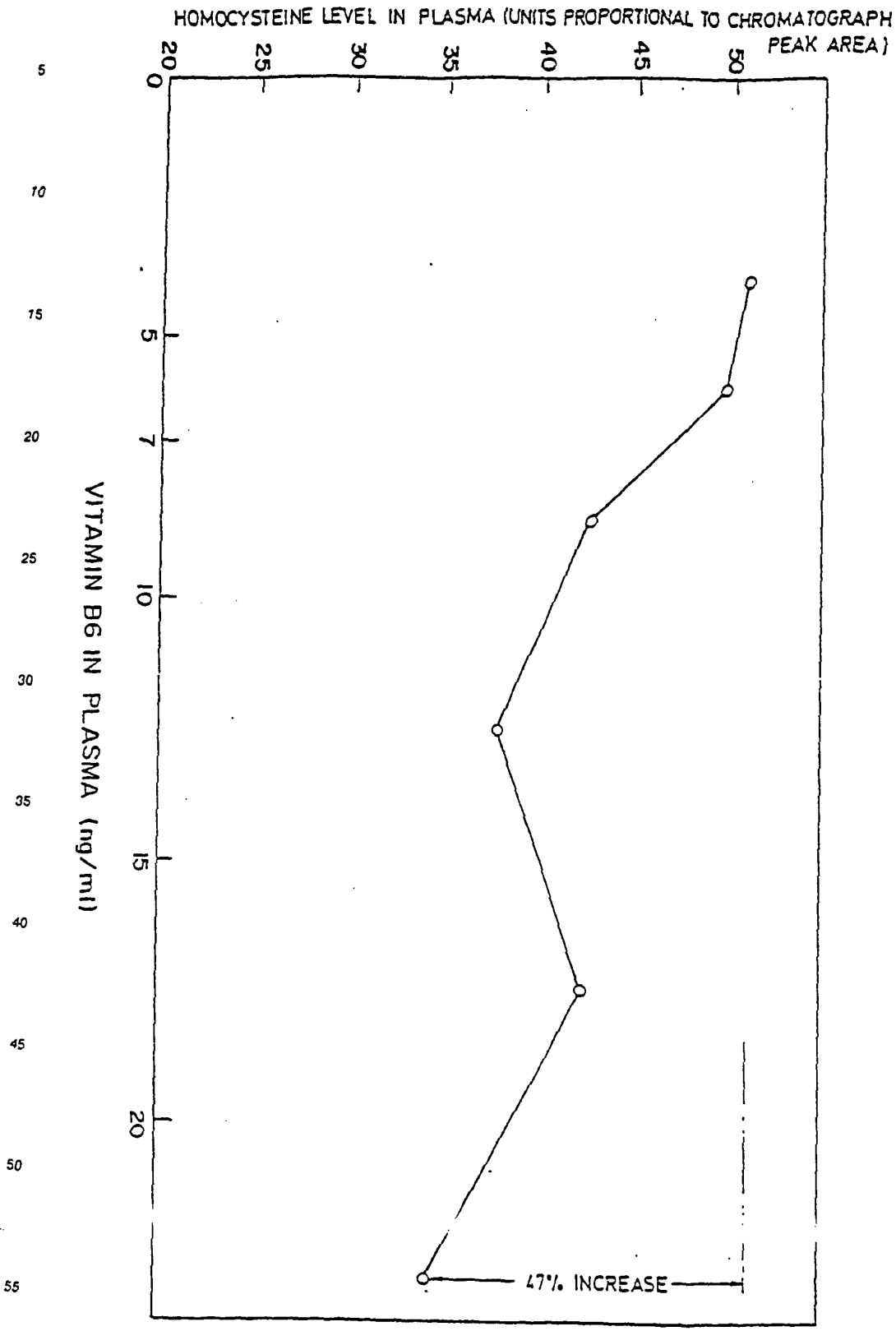
According to a sixth aspect of the invention, there is provided the use of a substance or composition capable of lowering, in plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, to inhibit atherosclerosis in the human body.

According to a seventh aspect of the invention, there is provided the use of a substance or composition capable of inhibiting, ie hindering or lowering, the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, to inhibit atherosclerosis in the human body.

The invention extends also to the use of both, or the combination of, said substance or composition capable of lowering the free sulfhydryl groups, and said substance or composition capable of inhibiting the deleterious disulfide exchange reaction, to inhibit atherosclerosis in the human body.

According to a eighth aspect of the invention, there is provided a method of inhibiting atherosclerosis in a human body, which comprises administering to the body an effective amount of a substance or composition capable of lowering the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules in the body plasma, thereby to inhibit directly atherosclerosis in the body.

According to a ninth aspect of the invention, there is provided a method of inhibiting atherosclerosis in


HOMOCYSTEINE LEVEL IN PLASMA (UNITS PROPORTIONAL TO CHROMATOGRAPH PEAK AREA)
20
25
30
35
40
45
50
0
5
7
10
15
20
VITAMIN B6 IN PLASMA (ng/ml)
5
10
15
20
25
30
35
40
45
50
55
47% INCREASE

of a carefully constructed standard curve. A special short program for the separation of homocysteine was developed, with plasma homocysteine yields that are in good agreement with the capillary gas chromatography -mass spectroscopy technique developed recently.

Claims

1. As an anti-atherogenic agent, a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

2. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises at least one oxidizing agent and/or an oxidizing promotion/catalyzing agent.

3. An anti-atherogenic agent according to Claim 2, characterized in that the substance or compound is selected from a cupric salt, a zinc salt, vitamin B_2, niacin, dihydroascorbic acid, quinone, a quinone derivative, a bioflavonoid compound, hesperidin, a steroid hormone, and a steroid hormone derivative.

4. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises at least one disulfide compound of the formula X-S-S-Y where X-SH and Y-SH are the same or different and are each a sulfhydryl containing compound, with X-S-S-Y being capable of reacting with homocysteine and/or cysteine in the human body to form mixed disulfides.

5. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises a compound X-SH and/or Y-SH, where X and Y are the same or different, and are capable of forming the disulfide compound X-S-S-Y within the body, with X-SH, Y-SH and X-S-S-Y being capable of reacting with homocysteine and/or cysteine in the human body to form mixed disulfides.

6. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises at lease one compound capable of decreasing homocysteine and/or cysteine levels per se in plasma, by its function as a co-factor or co-substrate in the transsulfuration pathway of methionine metabolism.

7. An anti-atherogenic agent according to Claim 6, characterized in that the compound is selected from Vitamin B_6, Vitamin B_{12}, folic acid, betaine, choline, or their metabolic precursors.

8. As an anti-atherogenic agent, a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby inhibiting directly the formation of atherosclerotic plaques in the himan body.

9. An anti-atherogenic agent according to Claim 8, characterized in that the substance or composition comprises at lease one compound capable of reacting directly or indirectly with the arterial wall cells and/or with the structural/functional proteins of the arterial wall cell-cement, thereby to reduce directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds of the structural/functional proteins.

10. An anti-atherogenic agent according to Claim 9, characterized in that the compound is selected from magnesium chloride, a bioflavonoid, a vasodilator, and an arterial cell relaxation agent.

11. As an anti-atherogenic agent, the combination of (i) a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, and (ii) a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby to inhibit directly the formation of atherosclerotic plaques in the human body.

12. The use of a substance or composition capable of lowering, in body plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby to inhibit directly atherosclerosis in the human body, for the manufacture of a pharmaceutical to inhibit atherosclerosis.

13. The use of a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby inhibiting directly atherosclerosis in the human body, for the manufacture of a pharmaceutical to inhibit atherosclerosis.

14. The use of a substance or composition capable of lowering, in body plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, to inhibit atherosclerosis in the human body.

15. The use of a substance or composition capable of inhibiting the deleterious disulfide exchange reaction or homocysteine and/or cysteine with structural/functional proteins in arterial walls, to inhibit atherosclerosis in the human body.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 347 864 A3**

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **89111246.8**

(22) Date of filing: **20.06.89**

(51) Int. Cl.5: **A61K 33/34**, A61K 33/30, A61K 31/525, A61K 31/455, A61K 31/375, A61K 31/105, A61K 31/095, A61K 31/68, A61K 31/505, A61K 31/205, A61K 31/00

(30) Priority: **24.06.88 ZA 884556**

(43) Date of publication of application: **27.12.89 Bulletin 89/52**

(84) Designated Contracting States: **AT BE CH DE ES FR GB GR IT LI LU NL SE**

(88) Date of deferred publication of the search report: **01.04.92 Bulletin 92/14**

(71) Applicant: **Strydom, Andries Johannes Cornelus**
21 Dersley Street, Bayswater
Bloemfontein, Orange Free State(ZA)

(72) Inventor: **Strydom, Andries Johannes Cornelus**
21 Dersley Street, Bayswater
Bloemfontein, Orange Free State(ZA)

(74) Representative: **Lewinsky, Dietrich, Dipl.-Ing., Dipl.-oec. publ. et al**
Patentanwälte Dipl.-Ing., Dipl.-oec. publ.
Dietriech Lewinsky Dipl.-Ing Reiner Prietsch
Gotthardstrasse 81
W-8000 München 21(DE)

(54) **Anti-atherogenic agents.**

(57) As an anti-atherogenic agent, a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

EP 0 347 864 A3

European Patent Office

EP 89 11 1246

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims:

☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: 1,2,3(partially),6,8,9,12-15

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

In view of the following documents: "Eur. J. Clin. Invest. 1978: HARKER et al.; Nature 1971, McCully et al.; J. Biol. Chem. 1987, Heinecke et al.; and Proc. Soc. Exp. Biol. Med. 1966, Renaud", which describe the general common inventive concept and especially in view of JP-A-57 197 284 which describes the use of cupric salts in oxidative processes and for treating atherosclerosis, the general inventive concept of claims 1 and 2 linking the compounds is no longer valid. Therefore the application contains the various following subjects:

1. Claims 1,2,3(partially),6,8,9,12-15: Use of a substance or composition capable of lowering the concentration of free sulfhydryl groups of homocysteine/cysteine or decreasing the levels of homocysteine/cysteine per se, or reacting with the functional or structural proteins of the arterial wall cells, in the manufacture of a medicament for treating atherosclerosis.

2. Claim 3(partially): Use of a zinc salt in the manufacture of a medicament for treating atherosclerosis.

3. Claims 3,7(partially): Use of vitamine B2, B6 or B12, niacin, folic acid or dihydroascorbic acid in the manufacture of a medicament for treating atherosclerosis.

4. Claim 3(partially): Use of a quinone or a derivative thereof in the manufacture of a medicament for treating atherosclerosis.

5. Claims 3,10(all partially): Use of a bioflavonoid, especially hesperidin in the manufacture of a medicament for treating atherosclerosis.

6. Claim 3(partially): Use of a steroid hormone or a derivative thereof in the manufacture of a medicament for treating atherosclerosis.

7. Claim 4: Use of a substance or composition comprising at least one disulfide compound of the formula X-S-S-Y wherein X and Y may be the same or different and may contain sulfhydryl groups capable of reacting with homocysteine and/or cysteine in the human body to form mixed disulfides, in the manufacture of a medicament for treating atherosclerosis.

./.

Obscurities

Claims searched incompletely: 1-3,6,8,9,12-15:

1. In accordance with the EP Guidelines for the searching division Chap. III, par. 3.6 (part B), the search has covered the subject matter to which the claims might reasonably be expected to be directed after they have been amended, namely: "The use of a substance or composition capable of a) lowering in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaque in the human body, or b) decreasing homocysteine and/or cysteine levels per se in plasma, by its function as a co-factor or co-substrate in the transsulfuration pathway of methionine metabolism, or c) inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial wall, thereby inhibiting directly the formation of atherosclerotic plaques in the human body or reducing directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds on the functional/structural proteins, in the manufacture of a medicament for treating atherosclerosis".

2. Neither a pharmacological mechanism (e.g. interaction with certain internal molecules (e.g. homocysteine/cysteine) or proteins (of the arterial wall)), nor a chemical reactivity (type of reaction taking place in situ)(or co-factor of an enzymatic reaction formation of disulfide bonds) can sufficiently describe the chemical structure of the formulas meant in the claims. The broadness and the vagueness concerning the compounds does not allow a meaningfull search.